



US005681932A

[11] Patent Number: 5,681,932

[45] Date of Patent: Oct. 28, 1997

- Gaynor, et al, 1984, *Proc. Natl. Acad. Sci. USA* 81: 1193-1197.
Alwine, 1985, *Mol. and Cell. Biol.* 5(5): 1034-1042.
Lewis and Manley, 1985, *Nature* 317: 169-171.
Lebkowski, et al, 1985 *Nature* 317:169-171.
Weeks and Jones, 1985, *Nuc. Acids Res.* 13(14) 5389-5402.
Leff and Chambon, 1986, *Mol. and Cell. Biol.* 6(1): 201-208.
Yan, et al, 1987, *Fed. Proc.* 46 2243.
Grinnell, et al 1986 abstract from Tumor Virus Meeting, Cold Spring, Harbor.
Grinnell, et al, 1986, *Mol. Cell. Biol.* 6(11):3596-3605.
Grinnell, et al 1987, abstract from 17th Steenbock Symposium.
Foster, et al. 1987, *Biochemistry* 26: 7003-7011.
Kumar, et al, 1986 *Proc. Nat. Acad. Sci.* 83:3199-3203.
Spangler, et al, 1987, *Science* 237:1044-1046.
Alonso-Caplen et al, 1987, abstract from Translational Control Meeting.
Hauschka, et al, 1986, *Haemostasis* 16:273-287.
Choo et al, 1986, *Gene* 46:277-286.
Sastry, et al, 1985, *Biochem Biophys. Res. Comm.* 132:795-803.
Christman, et al, 1982, *Proc. Natl. Acad. Sci. USA* 79:1815-1819.
Deschatnnette, et al, 1985, *Proc. Natl. Acad. Sci. USA* 82:765-769.
Hau, L. and Salem, H., 1988, *Thrombosis and Haemostasis* 60:267-270.
Grinnell, et al, 1987 *BioTechnology* 5:1189-1192.
Kawasaki, et al 1976, *J. Biol. Chem.* 251:1296-1302.
Goto, et al, 1988 *BioTechnology* 6:67-71.
Foster, et al. (1984) *Proc. Natl. Acad. Sci. USA* 81: 4766-4770.
Kisiel, *J. Clin. Invest.*, vol. 64, 1979, pp. 761-769.

Primary Examiner—James Ketter
Attorney, Agent, or Firm—Douglas K. Norman

[57] ABSTRACT

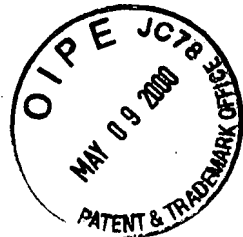
SA The present invention is a method of using the BK enhances in tandem with a eukaryotic promoter to promote transcription of DNA that encodes a useful substance. The method of the present invention requires the presence of the E1A gene product for maximum expression of the useful substance. The present invention also comprises a number of useful expression vectors that comprise the BK enhancer in tandem with the adenovirus 2 late promoter positioned to drive expression of a variety of proteins, such as protein C, chloramphenicol acetyltransferase, and tissue plasminogen activator. The present invention further comprises a method for increasing the activity of the BK enhancer involving placement of the BK enhancer immediately upstream of the eukaryotic promoter used in tandem with the BK enhancer to drive expression of a useful substance. Furthermore, the present invention also comprises a method for coamplification of genes in primate cells. Additionally, the invention further comprises the recombinant human protein C molecule produced in 293 cells which comprises novel glycosylation patterns.

(4 Claims, 42 Drawing Sheets)

EA

United States Patent [19]

Grinnell



[54] **METHOD OF USING EUKARYOTIC
EXPRESSION VECTORS COMPRISING THE
BK VIRUS ENHANCER**

[75] Inventor: **Brian W. Grinnell, Indianapolis, Ind.**

[73] Assignee: **Eli Lilly and Company, Indianapolis,
Ind.**

[21] Appl. No.: **458,372**

[22] Filed: **Jun. 2, 1995**

Related U.S. Application Data

[62] Division of Ser. No. 208,930, Mar. 9, 1994, which is a continuation of Ser. No. 368,700, Jun. 20, 1989, abandoned, Continuation-in-part of Ser. No. 250,001, Sep. 27, 1988, abandoned, Continuation-in-part of Ser. No. 129,028, Dec. 4, 1987, abandoned, Continuation-in-part of Ser. No. 849,999, Apr. 9, 1986, abandoned.

[51] Int. Cl.⁶ **C07K 14/745**

[52] U.S. Cl. **530/381; 435/240.2**

[58] Field of Search **435/69.1, 240.2,
435/172.3, 320.1, 226; 530/381**

[56] References Cited

U.S. PATENT DOCUMENTS

4,562,155 12/1985 Ricciardi et al. 435/172.3
4,775,624 10/1988 Bang et al. 435/226
4,959,318 9/1990 Foster et al. 435/69.1
4,965,199 10/1990 Capon et al. 435/69.6

FOREIGN PATENT DOCUMENTS

85301617.8 9/1985 European Pat. Off. .
0191606 8/1986 European Pat. Off. .
0215548 3/1987 European Pat. Off. .
0245949 11/1987 European Pat. Off. .
0246049 11/1987 European Pat. Off. .
WO87/04722 8/1987 WIPO .

OTHER PUBLICATIONS

Yang and Wu, 1979, *Science* 206:456-462.
Seif, et al. 1979, *Cell* 18:963-977.
Rosenthal, et al, 1983, *Science* 222: 749-755.
Milanesi, et al, 1984, *Molecular and Cellular Biology* 4:8,
1551-1560.
Khoury and Gruss, 1983, *Cell* 33:313-314.
Borrelli, et al, 1984 *Nature* 312:608-612.
Velcich and Ziff, 1985 *Cell* 40: 705-716.
Hen, et al, 1985 *Science* 230: 1391-1394.
Wood, et al, 1984, *Nature* 112: 330-337.
Zain, et al, 1979, *Cell* 16: 851-861.
Solnick, 1981 *Cell* 24: 135-143.
Berkner and Sharp, 1985, *Nuc. Acids Res.* 13(3): 841-857.
Kaufman, 1984, *Proc. Natl. Acad. Sci. USA* 82:689-693.
Mansour, et al, 1985 *Proc. Natl. Acad. Sci. USA* 82:
1359-1363.
Davis et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:
7560-7564.
Wasyluk, et al 1983, *Cell* 32: 503-514.
Imperiale, et al, 1983, *Cell* 35: 127-136.
Green, et al, 1983, *Cell* 35: 137-148.